Case Report Rapport de cas

Use of a tilapia skin xenograft for management of a large bite wound in a dog

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Abstract — A 13-year-old neutered male miniature dachshund suffered ~30% total skin loss following an attack by another dog. After numerous failed attempts at wound management and closure, the wound was successfully healed by epithelialization using tilapia skin grafts. At each tilapia skin graft placement, the wound bed appeared pink, clean, and healthy with excellent progression of epithelialization at all edges. With use of the tilapia grafts, epithelialization occurred at a rate of 1.76 mm/day. As a result, the wound reached complete closure by epithelialization with no evidence of wound contracture in 102 days.

Key clinical message:
Tilapia skin grafts were successfully used for management of a large bite wound in a dog and may promote accelerated epithelialization in full thickness skin wounds.

Résumé — Utilisation d'une xénogreffe de peau de tilapia pour la prise en charge d'une morsure importante chez un chien. Un teckel miniature mâle castré de 13 ans a subi une perte totale de peau d'environ 30 % à la suite d'une attaque par un autre chien. Après de nombreuses tentatives infructueuses de gestion et de fermeture de la plaie, la plaie a été cicatrisée avec succès par épithélialisation à l'aide de greffes de peau de tilapia. À chaque placement de greffe de peau de tilapia, le lit de la plaie apparaissait rose, propre et sain avec une excellente progression de l'épithélialisation sur tous les bords. Avec l'utilisation des greffes de tilapia, l'épithélialisation s'est produite à un taux de 1,76 mm/jour. En conséquence, la plaie a atteint une fermeture complète par épithélialisation sans signe de contracture de la plaie en 102 jours.

Message clinique clé :
Les greffes de peau de tilapia ont été utilisées avec succès pour la gestion d'une grande plaie de morsure chez un chien et peuvent favoriser une épithélialisation accélérée dans les plaies cutanées de pleine épaisseur.

(Traduit par Dr Serge Messier)

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Case description

A 13-year-old, 8.14-kg neutered male miniature dachshund dog was evaluated immediately after a traumatic attack by a dog. The dog had a reported history of hyperadrenocorticism, although records to support this diagnosis were not available for review nor was the dog on any current therapies. The dog was apparently otherwise healthy before the attack. Physical examination revealed multiple full thickness puncture wounds over the caudal abdomen with omentum extruding from the wounds. There were numerous puncture wounds and lacerations over the entire caudal dorsum with severe maceration of the skin, and large defects in the underlying subcutaneous tissues and pelvic musculature. A brief neurological evaluation revealed motor function and intact reflexes in all four limbs with intact cranial nerves. In-house blood chemistry analysis (Nova Biomedical Corporation, Waltham, Massachusetts, USA) was largely unremarkable. Lumbar spinal radiographs demonstrated severe subcutaneous emphysema with a large soft tissue defect dorsal to the iliac wings, but there was no evidence of spinal fracture or luxation.

The dog was transferred to the surgery department and emergency abdominal exploratory surgery revealed no internal

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organ damage. Incidentally, the liver appeared enlarged and liver biopsies were taken. Histopathological findings were consistent with vacuolar hepatopathy. The dog was then repositioned, and wounds on the dorsum were aseptically prepared for surgery. Necrotic skin was excised, and the underlying musculature was seen to be severely macerated, with substantial defects. Sharp debridement was performed until healthy bleeding tissue was encountered, resulting in 2 large circular shaped wounds: one over each hip, with an isthmus of healthy appearing tissue between the wounds along the dorsal midline. Culture of the wound bed was obtained, and a Vacuum-assisted Closure (VAC) system (Medela, McHenry, Illinois, USA) was applied over the wounds, maintaining at a pressure of −125 mmHg and changed every 3 to 4 d as per recommendations (1). Initially, the dog was administered ampicillin sodium/sulbactam sodium (Pfizer, New York, New York, USA), 50 mg/kg body weight (BW), IV, q8h, but the culture results revealed *Pseudomonas aeruginosa,* susceptible to marbofloxacin, so the antibiotic was changed to marbofloxacin (Zoetis, Parsippany-Troy Hills, New Jersey, USA), 3.2 mg/kg BW, PO, q24h for 21 d. On the second VAC change (6 d after the attack), a healthy bed of granulation tissue had formed, but the isthmus of tissue between the 2 wound beds had become necrotic. This tissue was sharply excised, the wound on the left side was closed by primary intention, and a closed suction drain was placed. The wound on the right side was partially closed with Manuka honey and a tie-over bandage. On the second bandage change, partial dehiscence of the left incision was detected. The drain was removed, necrotic skin edges were debrided, and the site managed with tie-over bandages. During this time, the dog's comfort and attitude began to improve markedly, discharge from the wound lessened, and the tissue became very vibrant and healthy in appearance. At this time, (16 d after initial presentation) an advancement flap was performed to assist in wound closure. This technique was selected to help address the large size of the wound, the expected prolonged healing, and the risk of wound contracture. With careful planning, advancement flaps provide cutaneous circulation with extensive connections between vascular elements and excellent collateral flow to adjacent areas of the skin with low risk of donor tissue necrosis (2). The advancement flap moved skin from the cranial dorsum, caudally, to cover the wound. The inguinal flank folds were also rotated to cover the lateral-most aspect of the wound on both sides. Two days after surgery, the flap became erythematous and congested, but appeared viable. Hirudotherapy was attempted twice in hopes of relieving venous congestion of the flap, but the leeches (Leeches USA, Westbury, New York, USA) would not attach (3). Four days after surgery, a small area of necrosis developed at the right edge of the flap. A tie-over bandage was placed, and the dog was discharged 22 d after the attack with a fentanyl transdermal patch (Janssen Pharmaceuticals, Titusville, New Jersey, USA), 25 μg and gabapentin (Amneal Pharmaceuticals, Bridgewater Township, New Jersey, USA), 12.2 mg/kg BW, PO, q8 to 12h.

Postoperative plans were to recheck the dog and change the bandage twice weekly, and to consider additional wound closure once healing from the advancement flap surgery had occurred. At recheck 24 d after the attack and 2 d after discharge, 80% necrosis of the flap was detected (Figure 1). The following day, the necrotic flap was debrided, a repeat culture was performed, and the wound was managed with negative pressure wound therapy. A complete blood (cell) count (CBC) and chemistry analysis done at this time revealed a serum alkaline phosphate of 2037 IU/L [reference range (RR): 5 to 131 IU/L], serum aspartate transaminase of 197 IU/L (RR: 15 to 166 IU/L), and serum creatinine phosphokinase of 1807 IU/L (RR: 59 to 895 IU/L). Elevated liver enzymes were consistent with the dog's liver histopathology and the history of suspect hyperadrenocorticism, but at this time the owner declined further testing to investigate these findings. Repeat culture revealed a methicillin-resistant *Staphylococcus pseudintermedius,* sensitive to meropenem, so meropenem (Novartis, Cambridge, Massachusetts, USA), 8.5 mg/kg BW, SC, q12h was administered for 16 d. While being managed with the VAC, the wound bed became very healthy in appearance, and granulation tissue formation was evident; however, the wound was not becoming noticeably smaller, and the dog was becoming progressively depressed and aggressive. It became apparent that other options needed to be considered to manage the wound on an outpatient basis. Given that the previous advancement flap had become necrotic causing further skin loss, there was concern about performing another flap. There was not enough remaining skin on the dorsum for another advancement flap, and the inguinal folds had already been used to close the lateral most aspect of the wounds. A caudal superficial epigastric axial pattern flap was considered, but also carried some risk of flap necrosis. Loss of any more skin would have been detrimental for this dog. Also, if the lesions were allowed to heal by second intention, severe wound contracture was of major concern. An additional challenge to management was that the dog and owners primarily lived abroad. Therefore, the potential use of an alternative skin graft was investigated. Tilapia skin grafts are known to be used in human and animal burn victims, but there were no commercial sources available to the authors. Thus, alternate options of procurement were explored.

Sixteen days after the second VAC system placement (36 d after the attack), tilapia skin grafts were prepared based on a procedure described by Costa et al (4). Cold sterilization has shown to effectively sterilize tilapia skin grafts, while preserving their 3-dimensional matrix, collagen, and omega-3 fatty acids (5). Fresh tilapia acquired from a local seafood market were skinned. Using sterile instruments in an aseptic environment, any remaining muscle and soft tissue were removed from each piece of skin using a #10 scalpel blade. Skins were copiously lavaged with sterile saline and placed in following order with sterile saline washes in between: 2% chlorhexidine for 90 min, 100% glycerol and 2% chlorhexidine at a 3:1 ratio for 60 min, manual massage with 100% glycerol for 5 min, and 100% glycerol for 60 min. After a final lavage, each skin was swabbed and submitted as a batch for an aerobic and anaerobic culture. Each piece was individually packaged into a sterile plastic sleeve (KARL STORZ SE & Co. KG, Tuttlingen, Germany) with ends securely sealed and deposited into a paper-plastic peel sterilization pouch (Propper Manufacturing, Long Island City, New York, USA) for protection and convenience for storage at

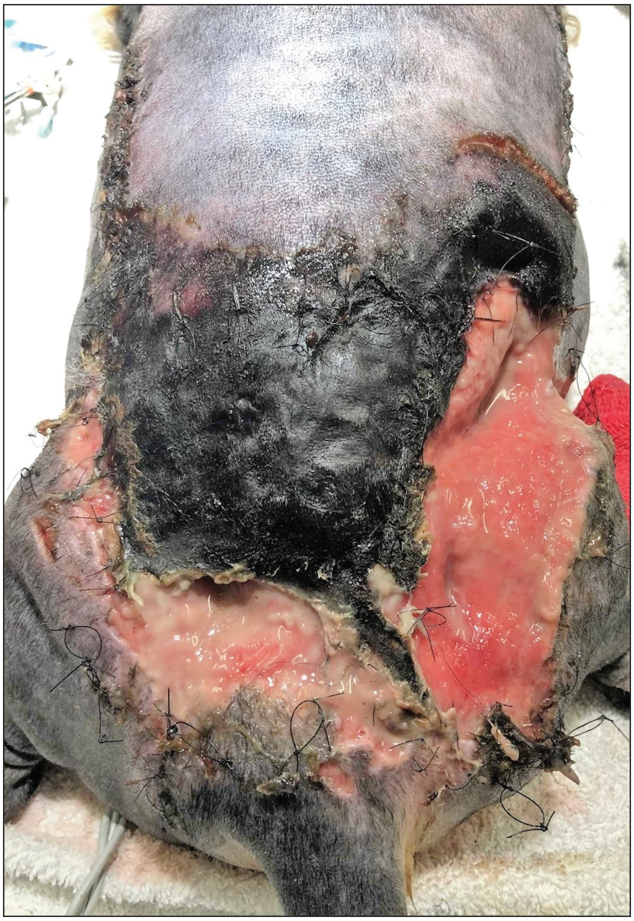

Figure 1. Eighty percent necrosis of advancement flap with significant purulent discharge noted 9 d after surgery.

4°C. When both anaerobic and aerobic cultures were negative and finalized, the skins were made available for use.

In preparation for the procedure, the dog was pre-medicated with methadone (Henry Schein, Melville, New York, USA), 0.4 mg/kg BW, IV. Anesthesia was induced with ketamine (Vet One, Las Vegas, Nevada, USA), 2 mg/kg BW, IV, fentanyl, 4 μg/kg, IV and midazolam (Roche Company, Basel, Switzerland), 0.4 mg/kg BW, IV. The dog was then endotracheally intubated, and anesthesia was maintained with isoflurane (Vet One). The dog was placed in sternal recumbency, the VAC system was removed, and the caudal dorsum and wound region were aseptically prepared and draped. Exuberant granulation tissue was sharply excised, and the skin-granulation bed interface was incised to expose healthy, bleeding tissue edges. The wound bed measured 15 (cranial to caudal) × 15 (medial to lateral) × 18 cm (left cranial to right caudal). The wound bed was copiously lavaged with sterile saline and a 3rd aerobic culture was obtained. Tilapia skin grafts were aseptically retrieved from their sterilant package and laid over the wound bed and secured with a cruciate pattern using 4-0 Ethilon (Ethicon, Somerville, New Jersey, USA). The tilapia skin graft was also kept moist with application of hydrogel amorphous wound dressing (Covidien, Mansfield, Massachusetts, USA) using gloves, q2 to 6h. A third aerobic culture from the site revealed growth of methicillin-resistant *S. pseudintermedius* and *Enterococcus* spp., which were sensitive to chloramphenicol. Meropenem, therefore, was discontinued and chloramphenicol was added to the dog's medical regimen. The dog remained hospitalized with gabapentin (Amneal Pharmaceuticals, Bridgewater Township, New Jersey, USA), 10 mg/kg BW, PO, q8h and chloramphenicol (Bimeda, Oakbrook Terrace, Illinois, USA), 55 mg/kg BW, PO, q8h for 28 d.

Despite attempts to keep the graft moist, the tilapia skin graft became very desiccated and was not incorporating into the wound bed as expected based on previous reports (4,6,7). Five days after the first tilapia skin graft placement, it was replaced with a second tilapia skin graft. At this time, the wound bed was pale tan with exudate present (Figure 2A). The wound bed was copiously lavaged with sterile saline, TrizEDTA (Dechra Veterinary Products, Overland Park, Kansas, USA) and amikacin, and debrided appropriately. Tilapia skin grafts were placed and to help combat desiccation of the grafts, hydrocolloid foam dressings were trimmed to fit the wound and secured with a tie-over bandage.

In 4 d, the tilapia graft was clean, moist, and intact with mild clear, serosanguinous discharge on the bandaging material. The graft appeared thin and translucent, and portions of the graft were no longer present. After removal, the wound bed appeared pink and healthy. Epithelialization was evident around the wound edges, and the wound was an estimated 25% smaller (14 × 13 × 15 cm). As multiple veterinarians were involved in this case, a different approach was considered at this time due to clinician's preference. A Procellera dressing (Arthrex Vet Systems, Fort Myers, Florida, USA) with hydrogel was applied over the wound rather than new tilapia grafts and covered with telfa pads and a laparotomy pad secured with a tie-over bandage.

Six days later, there was a clear deterioration of the health of the wound and the dog was assessed as being more painful. Brown discharge on the Procellera bandage was noted with moderate crusting. The granulation bed appeared pale, the wound was coated with thick, fibrinous debris, and the wound edges were more erythematous compared to the previous bandage change. The wound edges and flanks were more taut, suggesting some wound shrinkage by contracture. At this time of evaluation, the wound bed measured at 12.5 × 12 × 14 cm. Further tilapia skin grafts were placed in similar fashion as described. The dog's comfort and demeanor improved, and he was discharged the following day (54 d after the attack) with gabapentin, 100 mg, PO, q12h and chloramphenicol, 250 mg, PO, q8h for 14 d. The wound was managed on an outpatient basis thereafter.

The dog was re-evaluated 13 d after the last tilapia graft was placed. During the dog's stay at home, the previous tie-over bandage fell off and was replaced with a soft padded wrap with calcium alginate sheets and hydrocolloid foam. At that time, photographs of the wound revealed a healthy appearance with progressive epithelialization. Upon evaluation at the clinic 3 d later, the wound bed (9 × 10 × 10 cm) appeared very pink, clean, and healthy with progression of epithelialization at all edges with no evidence of remaining tilapia graft or further

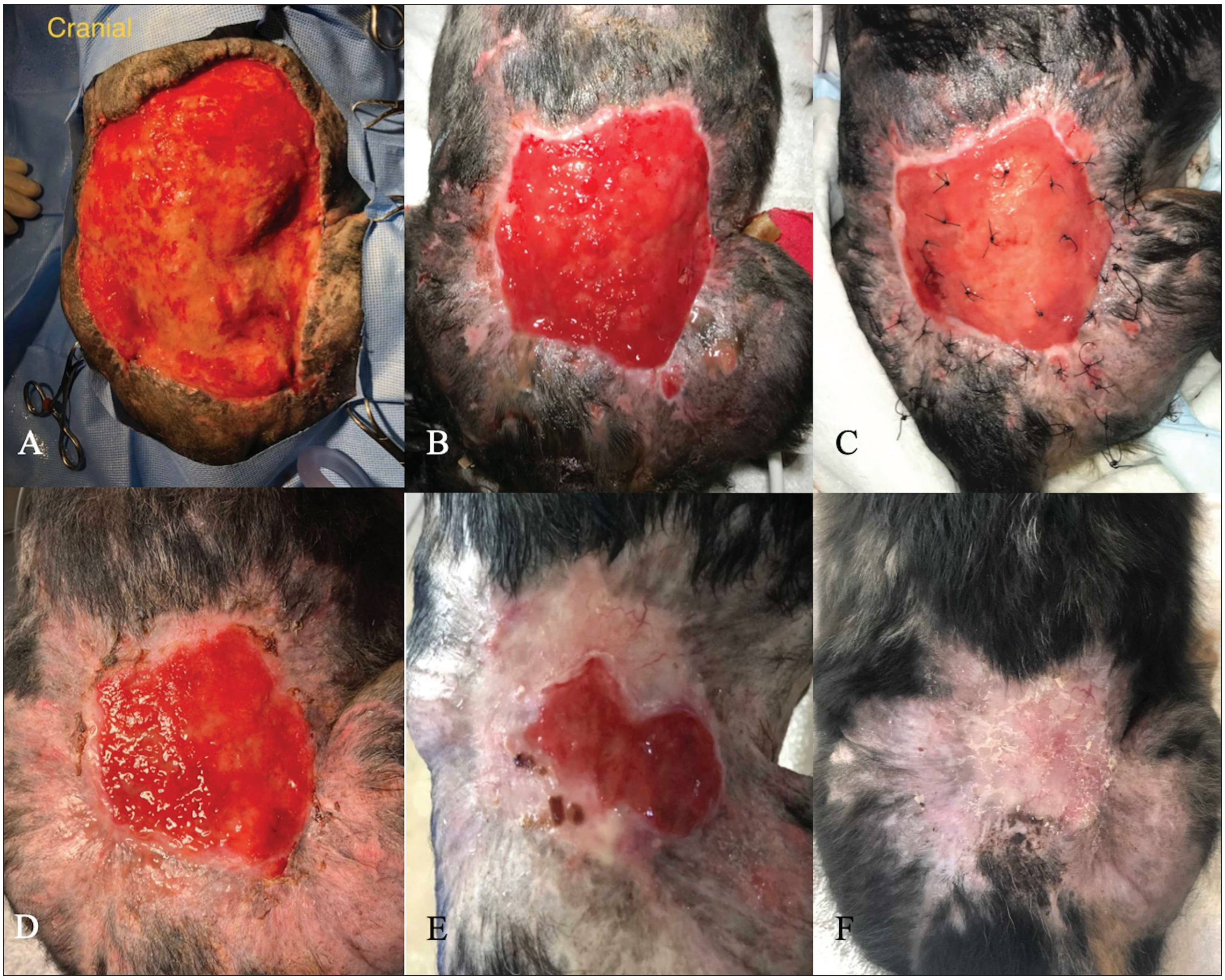

Figure 2. Progression of wound epithelialization with use of tilapia skin grafts, 5 d after initial tilapia skin graft placement – June 15, 2019 (A). Twelve days after the 4th tilapia skin graft placement – July 7, 2019 (B). Fourteen days after the 5th tilapia skin graft placement – July 21, 2019 (C). Twenty-one days after the 6th tilapia skin graft placement – August 11, 2019 (D). Thirty-four days after the 7th (final) tilapia skin graft placement – September 14, 2019 (E). Fifty-eight days after the 7th (final) tilapia skin graft placement – October 18, 2019 (F).

contracture (Figure 2B). Tilapia skin grafts were placed and covered as previously described.

After 14 d, the wound bed (7 × 9 × 8.5 cm) appeared pink and healthy with excellent progression of epithelialization at all edges (Figure 2C). Tilapia skin grafts were repeated as previously described.

After 21 d, the wound bed (5 × 5.5 × 6.5 cm) remained healthy in appearance, and epithelialization continued to progress (Figure 2D). A tilapia skin graft was placed over the wound with hydrocolloid foam dressing and 2 Primapore bandages (Primapore; Smith & Nephew, London, United Kingdom). With the owner's approval, a 2 cm × 5 mm skin biopsy, including normal skin, epithelialized skin, and the active granulation tissue, was retrieved, and submitted for analysis to gain more information on wound healing at a microscopic level. Figure 3 is a dermatohistopathologic review of the healing wound that revealed healthy granulation tissue in the dermis and wound epithelialization. Some sections also showed near-healing erosions and ulcers with crusted superficial suppurative inflammation with no signs of overlying graft remaining.

The owner provided a photograph 14 d after the last graft placement. The wound had decreased in size and measured approximately 2 cm (Figure 2E). The owner was instructed to apply calcium alginate sheets and change the bandage as needed until complete healing occurred. The owner provided photographic documentation of the completely epithelialized wound, with minimal to no appreciable contracture, 161 d after attack and 103 d after the first tilapia graft placement (Figure 2F).

Discussion

Tilapia skin grafts have been used successfully for management of various types of wounds in human medicine including burn wounds, diabetic ulcers, and traumatic wounds sustained in combat (4,6–8). In human studies, tilapia skin grafts have been shown to be superior to other wound dressings. Although the accelerated wound healing properties conferred by tilapia skin

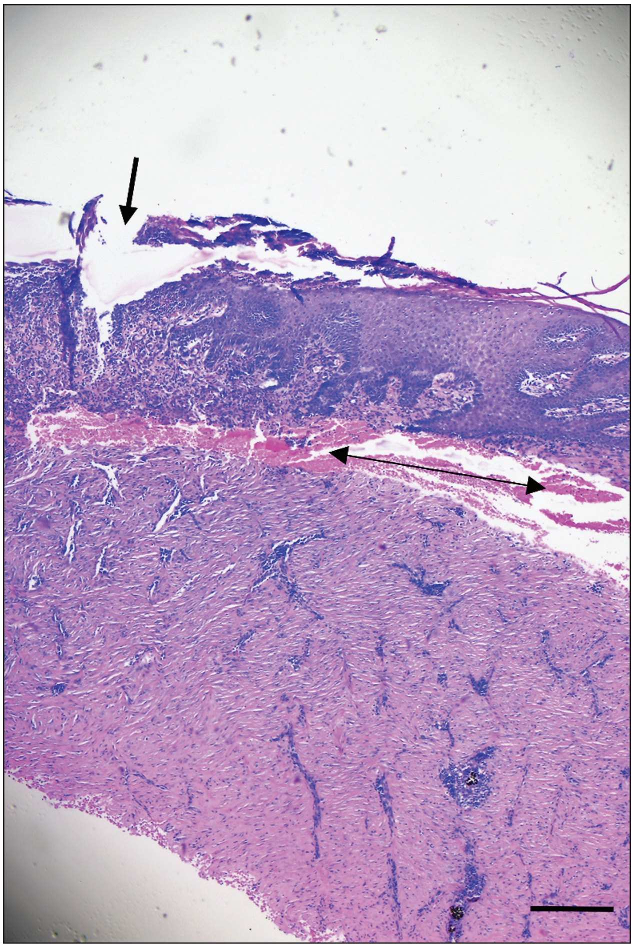

Figure 3. Dermatohistopathology after the tilapia graft showing re-epithelization and mature granulation tissue. Focal healing erosion and ulcer with crusted superficial suppurative inflammation remaining (single arrow). Artifactual cleft in the dermis with hemorrhage was an artifact from procurement and processing (double arrow). Image taken on September 9, 2019. H&E stain; bar = 300 μm.

grafts is not fully understood, documented benefits include its porous 3-dimensional structure, rich omega-3 polyunsaturated fatty acid (PUFA) concentration, anti-bacterial properties, high collagen concentration, and its cost-effectiveness and accessibility (4,6,9,10). Due to its unique structural and biochemical properties, studies have even demonstrated tilapia skin grafts' superiority over other dressings such as human amnionic and chorionic membranes and porcine small-intestine submucosa (9,11). Tilapia skin provides a highly porous and robust extracellular matrix, which creates an environment for superior cell migration and keratinocyte ingrowth, thereby promoting accelerated epithelialization (6,9,10). Tilapia skin is also rich in omega-3 PUFAs, eicosapentaenoic acid, and docasahexaenoic acid which are known to be highly effective in modulating the inflammatory response of wound healing (12,13). Not only has omega-3 PUFA shown anti-inflammatory effects, but also may play an important role in withstanding bacterial invasion. Magnusson et al (9) showed that tilapia skin can act as a potential bacterial barrier for up to 48 to 72 h and its bacterial barrier properties were augmented when the fish skin graft was supplemented with oral omega-3 PUFA. Further investigation is required to demonstrate how tilapia skin can directly have anti-bacterial properties, but studies have shown that omega-3 fatty acids can possess antiviral and antibacterial properties (14,15). With all of these advantages in mind, tilapia skin grafts can potentially be an innovative alternative resource for wound healing.

With the use of a tilapia skin graft, we demonstrated complete healing of a large bite wound with rapid re-epithelialization, and minimal to no appreciable wound contracture in approximately 3 mo. Complete healing of a primarily closed wound can reach epithelial seal within 24 h as re-epithelialization normally occurs at an approximate rate of 1 mm/d (16). Based on the largest diagonal distance of 18 cm measured from left cranial to right caudal, complete epithelialization was reached at an approximate rate of 1.76 mm/d. In addition, histopathologic imaging of the wound bed reveals incorporation of the tilapia skin graft into the wound, as it is converted into a functional living tissue, while breaking down in an environment evidenced with infection. This suggests that tilapia skin grafts not only promote rapid healing but may do so even in the presence of superficial bacterial contamination. The infrequency of follow-up care was a challenge in this case. International travel of the dog and client interfered with the ability to recheck the patient once discharged. Based on the experiences from this case, the authors suggest that new tilapia skin graft placements can range from every 3 to 14 d, depending on the dog's stability, owner compliance, and the degree of exudation from the wound, which may necessitate more frequent bandage care. The authors also recommend covering the tilapia graft with absorbent and non-adherent bandaging material to prevent graft desiccation.

Although the dog was not diagnosed with hyperadrenocorticism, it would have been beneficial to have definitively investigated that condition as this may have a negative effect on wound healing (17). Hyperadrenocorticism could have played an influential role in the dog's healing process. If tilapia skin grafts are effective in healing wounds in patients with hyperadrenocorticism, then this information could potentially help patients with chronic diseases and having poor healing abilities. Further research is required to support this theory.

In recent human literature, tilapia skin grafts have been shown to be safe and effective, with superior wound healing properties, and their use was easily adapted to this dog (6–9). Additional studies are needed to further investigate the utility of this technique in veterinary medicine. Although several anecdotal examples of the use of tilapia skin grafts have been reported in veterinary medicine, to the best of the authors' knowledge, this is the first description of the use of tilapia skin for a dog bite wound in a refereed journal. Tilapia skin grafts can be an effective, cost-efficient method for wound management and may benefit patients with many types of wounds and in multiple clinical scenarios. CVJ

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